PHYLOGEOGRAPHY OF THE NORTHERN YELLOW BAT (*LASIURUS INTERMEDIUS*) SUBSPECIES IN TEXAS

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Abstract

Northern Yellow Bats (*Lasiurus intermedius*) exist in the southeastern United States and the southeastern counties of Texas along the coast of the Gulf of Mexico and extend into Mexico. The northern yellow bat is made up of two subspecies, *L. i. floridanus* and *L. i. intermedius*, which are differentiated by their size and the color of their pelage. There is a proposed line separating the two subspecies that goes through Atascosa County to Matagorda County. We used DNA sequence of the Cytochrome b gene to determine if there are two genetic lineages corresponding to the currently recognized subspecies. Samples were collected from individuals submitted to the Texas Department of State Health Services for rabies testing and supplemented with samples in the ASNHC. A total of 46 sequences originating in 12 counties and one sequence from an individual found in Mexico were included in a Maximum Likelihood analysis. Our results show that there were not two lineages recovered that correspond to the subspecies designations. We conclude that the specimens should be examined morphologically to confirm their subspecies designation and further molecular work should be conducted to test levels of gene flow across the range of this species.

Introduction

Northern Yellow Bats (*Lasiurus intermedius*) are naturally found in the southeastern United States, the southeastern counties of Texas, and parts of Central America. This species is made up of two subspecies, *L. i. intermedius* and *L. i. floridanus*, which are largely differentiated by the color of their pelage and their size. *L. i. intermedius* are typically larger and have a more intensely yellow pelage compared to *L. i. floridanus,* which are typically less than 13.5 cm in total body length (Ammerman et al. 2012). Adult females of both subspecies are typically found to be larger than adult males. The two subspecies are found in the southeastern Texas counties, but whether or not they are separated geographically is not specifically known. A line through Acosta County to Matagorda County has been proposed to separate the two subspecies geographically.

Objective

Our objective was to test the hypothesis that the morphologically defined subspecies of *L. intermedius* would form distinct lineages based on analysis of cytochrome b gene sequence.

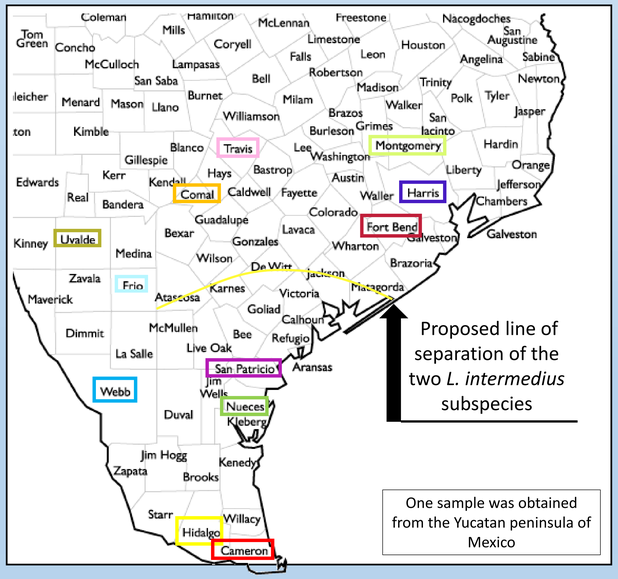

Figure 3 – Southeastern counties of Texas from which Northern Yellow Bat specimens originated. The yellow line through Atascosa county to Matagorda county shows the previously proposed line that separated *L. i. floridanus* and *L. i. intermedius* subspecies. A total of 47 samples were analyzed, including one sample obtained from the Yucatan peninsula.

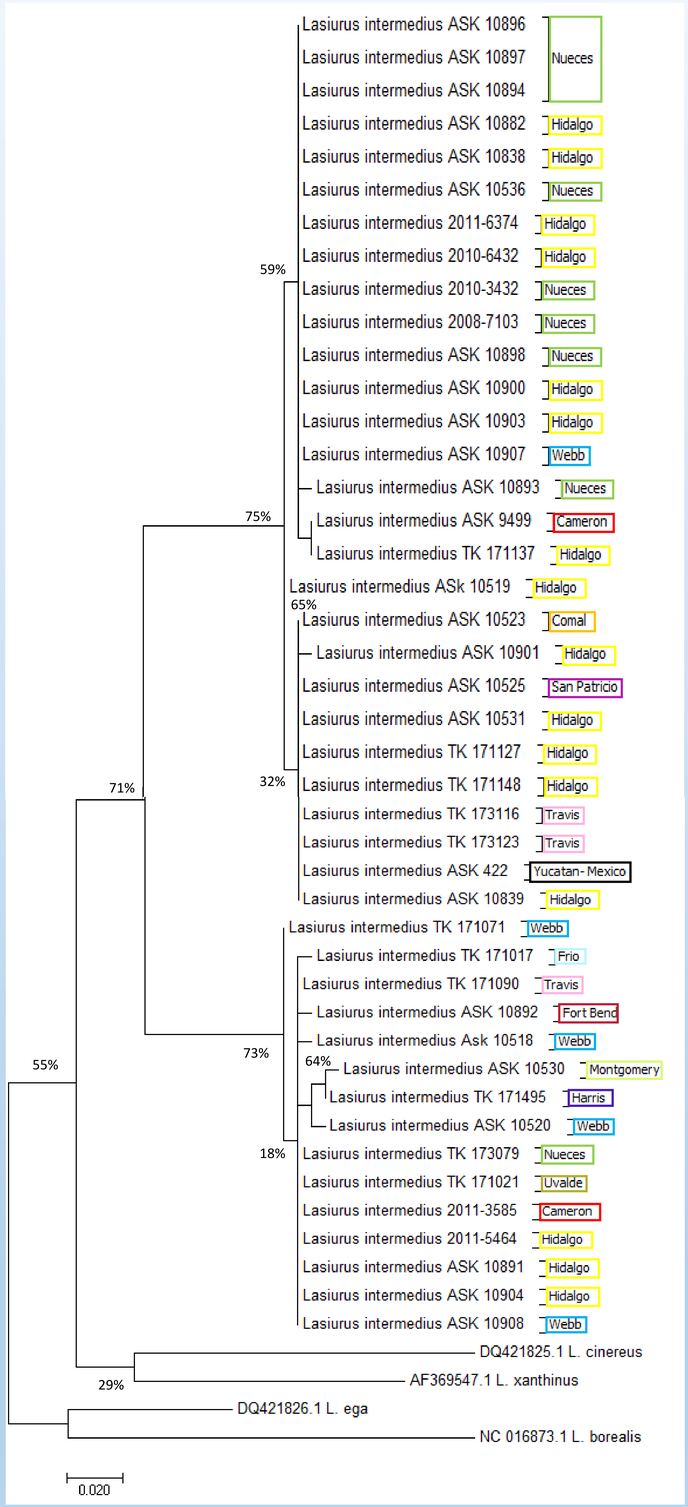

Figure 2 – A phylogenetic tree of 47 samples of the *Lasiurus* genus across 12 counties in Texas and the Yucatan peninsula. Analysis was performed using Maximum Likelihood using the GTR model of DNA substitution (likelihood score -812.7043). Bootstrap values based on 1000 replicates are shown at main branches.

Methods

A phylogenetic analysis was run with 47 DNA sequences from different individuals. DNA sequences were obtained from Genbank, previous research data, and tissue samples acquired from the Angelo State Natural History Collection. Tissue samples underwent gene amplification using either primer set MVZ04/MVZ05 or primers 14841 (Kocher et al. 1991) and 15547 (Edwards et al. 1991), targeting the cytochrome b gene, a mitochondrial gene passed through maternal lineages. The mitochondrial cytochrome b gene was chosen due to its 3rd codon site variability and its effectiveness in analyzing within a genus or species. PCR products were tested for purity and concentration, as well as visualized using gel electrophoresis. Products were cleaned and sent to the Texas A&M University Corpus Christi Genomics Core Lab for sequencing. Phylogenetic analysis was performed using Maximum Likelihood analysis (General Time Reversible model). This analysis tested 208 total nucleotide positions. A second analysis composed of a subset of the samples analyzed a total of 434 positions and the same pattern was generated in the phylogenetic tree. A bootstrap analysis was run with 1000 replicates.

Figure 1 – Photograph of a Northern Yellow Bat roosting. Photograph by Abbott Nature Photography, Austin, Texas.

Results and Discussion

Samples of *Lasiurus intermedius* were analyzed in a phylogenetic tree along with four samples from other species in the *Lasiurus* genus (Figure 2). The counties are color coded between Figure 3 and Figure 2, relating where specific samples originated. The Maximum Likelihood phylogenetic tree analysis (Figure 2) showed two distinct clades among the samples, although the two lineages did not correspond to geography. Bootstrap values are considered significant if greater than 70% (Hillis and Bull, 1993). This divergence indicates two distinct maternal lineages. Further analysis examining the morphology within the Northern Yellow bat species will provide more evidence for this particular study. From the molecular analysis, however, there is not significant support for the geographical separation of *Lasiurus intermedius intermedius* and *Lasiurus intermedius floridanus* across the proposed line from Atascosa county to Matagorda county. Both clades contain individuals from south and north of the proposed line. Continued studies should examine larger gene fragments and results should include larger bootstrap values for analysis of the sequences.

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